

IN THE CLAIMS:

Claim 1 (Currently amended) A method for producing a high yield of purified immune globulins from blood plasma, comprising:

providing a plasma source containing immune globulins;

suspending the immune globulins in an ethanol solution at a volume equivalent to two times that of the plasma source at a temperature in a range of about -4°C to -6°C;

adjusting the pH of the suspension to about 5.7 to 5.8;

incubating the suspension for at least two hours;

adding a volume of a solution of about 2.4M glycine in about 7% ethanol and purified water (volume/volume) equivalent to the volume of the plasma source to the suspension;

adjusting the pH of the suspension to about 5.2 to 5.4;

extracting the immune globulins from the suspension in a liquid phase to provide an extract containing immune globulins;

concentrating the extract containing immune globulins by ultrafiltration of the extract containing immune globulins to provide an immune globulin solution of approximately 1.0 gram/deciliter immune globulins content;

performing solvent-exchange on the immune ~~globulins~~ globulin solution with a sodium phosphate solution to provide an extract solution containing immune globulins;

removing any impurities from the extract solution containing immune globulins using an anion exchange chromatography column to provide a purified extract solution containing immune globulins;

concentrating the purified extract solution containing immune globulins deriving from an effluent of the anion exchange chromatography column by ultrafiltration to provide a concentrated purified extract solution containing immune globulins;

inactivating viruses present in the concentrated purified extract solution containing immune globulins;

passing the concentrated purified extract solution containing immune globulins through a column containing C-18 resin for removal by adsorption of any residue in the concentrated purified extract solution containing immune globulins from the step of inactivating viruses, wherein the ratio of immune globulins load volume to resin volume is approximately eight parts load volume to one part C-18 resin; and

formulating the ~~collected~~ concentrated purified extract solution containing immune globulins for final use.

Claim 2 (Previously presented) The method of claim 1, wherein the plasma source containing immune globulins is selected from the group consisting of a Cohn fraction II+III and a Cohn fraction I+II+III.

Claim 3 (Previously presented) The method of claim 1, wherein the ethanol solution is comprised of about 19% ethanol and about 81% purified water adjusted to a pH of 5.7 to 5.8.

Claim 4 (Previously presented) The method of claim 1, wherein the step of suspending the immune globulins in the ethanol solution comprises vigorously agitating the plasma source.

Claim 5 (Currently amended) The method of claim 1, wherein the ~~suspension of~~ step of suspending the immune globulins in the ethanol solution ~~preferably~~ occurs at a temperature of about -5°C.

Claim 6 (Original) The method of claim 1, wherein the plasma source containing immune globulins is derived from human blood plasma.

Claim 7 (Previously presented) The method of claim 1, wherein the plasma source containing immune globulins is derived from a non-human source.

Claim 8 (Previously presented) The method of claim 1, wherein the volume of the immune globulins suspension increases to a volume equivalent to three times that of the plasma source to enhance protein recovery.

Claim 9 (Original) The method of claim 1, wherein the plasma source contains phospholipids.

Claim 10 (Previously presented) The method of claim 9, wherein step of adjusting the pH of the suspension comprises adding 1M sodium acetate to effect precipitation of a majority of the phospholipids as the suspension is continuously agitated.

Claim 11 (Currently amended) The method of claim 9, wherein the step of adjusting the pH of the suspension comprises adding 4M sodium acetate to effect

precipitation of a majority of the phospholipids as the suspension is continuously agitated.

Claim 12 (Previously presented) The method of claim 1, wherein the step of incubating the suspension for at least two hours comprises moderately agitating the suspension.

Claim 13 (Original) The method of claim 1, wherein the step of adding the solution of glycine and ethanol to the suspension comprises vigorously mixing the suspension.

Claim 14 (Currently amended) The method of claim 1, wherein the step of adding the solution of glycine and ethanol to the suspension comprises producing a final concentration of glycine in the suspension of about 0.8M and a final concentration of ethanol in the suspension of about 15% (volume/volume).

Claim 15 (Previously presented) The method of claim 1, wherein the step of adjusting the pH of the suspension to about 5.2 to 5.4 further comprises increasing the suspension temperature to approximately -2°C to -3°C.

Claim 16 (Currently amended) The method of claim 1, wherein ~~liquid-solid separation~~ the step of extracting the immune globulins from the suspension is performed by one of centrifugation and filtration.

Claim 17 (Currently amended) The method of claim 1, wherein the step of extracting the immune globulins from the suspension is performed ~~preferably~~ by use of a filter press.

Claim 18 (Currently amended) The method of claim 1, wherein ~~liquid-solid separation~~ the step of extracting the immune globulins from the suspension is facilitated using diatomaceous earth at a concentration of about 1% to about 3% weight by volume during filtration.

Claim 19 (Currently amended) The method of claim 1, wherein the step of extracting the immune globulins from the suspension ~~extraction~~ is performed at a temperature in a range of about -2°C to -3°C while moderately agitating the suspension.

Claim 20 (Currently amended) The method of claim 1, wherein the extract ~~solution~~ containing immune globulins is concentrated by ultrafiltration at a temperature in a range of about -2°C to -3°C while moderately agitating the ~~protein-solution~~ extract containing immune globulins.

Claim 21 (Currently amended) The method of claim 1, wherein the extract ~~solution~~ containing immune globulins is concentrated through an ultrafilter membrane having a molecular weight cut off of about 100,000.

Claim 22 (Currently amended) The method of claim 1, wherein the step of performing solvent-exchange is performed using a solution of about 20mM sodium phosphate at a temperature of about 5°C and a pH of about 6.5.

Claim 23 (Currently amended) The method of claim 1, wherein the step of performing solvent-exchange is performed ~~by preparing a mixture of~~ using a solution of sodium phosphate prepared by mixing sodium phosphate monobasic and sodium phosphate dibasic at a ratio that yields a pH of about 6.5.

Claim 24 (Currently amended) The method of claim 1, wherein the step of performing solvent-exchange is performed using a solution of about 20mM sodium acetate at a pH of about 6.5.

Claim 25 (Currently amended) The method of claim 1, wherein the step of performing solvent-exchange is performed by addition of one volume of the pH 6.5 buffer to the ~~protein~~ immune globulins solution forming ~~a new protein solution~~ the extract solution containing immune globulins and concentrating the ~~new protein solution~~ extract solution containing immune globulins to its original volume.

Claim 26 (Currently amended) The method of claim 1, wherein the step of performing solvent-exchange is performed approximately four times to reduce the alcohol and glycine content.

Claim 27 (Previously presented) The method of claim 26, further comprising increasing the temperature of the immune globulins solution to room temperature at 15°C to 25°C after approximately the fourth solvent-exchange.

Claim 28 (Original) The method of claim 1, wherein the anion exchange chromatography column is equilibrated with a 20mM sodium phosphate buffer at a pH of about 6.5.

Claim 29 (Previously presented) The method of claim 28, wherein the anion exchange chromatography column is washed with at least one column volume of the 20mM buffer after passing the extract solution containing immune globulins therethrough to obtain further immune globulins recovery.

Claim 30 (Currently amended) The method of claim 1, wherein the anion exchange chromatography column is packed with a gel, and a ratio of immune globulins to the ~~column gel~~ is approximately 0.4 grams of protein/milliliter of ~~packed column gel~~.

Claim 31 (Currently amended) The method of claim 1, wherein the purified ~~purified~~ extract solution containing immune globulins is concentrated from the column effluent to approximately 6 grams/deciliter immune globulins using an ultrafilter membrane having a molecular weight cut off of about 100,000.

Claim 32 (Currently amended) The method of claim 1, wherein a solvent-detergent method is used for inactivating viruses present in the concentrated ~~protein~~ purified extract solution containing immune globulins.

Claim 33 (Previously presented) The method of claim 32, wherein a mixture of the purified extract solution containing immune globulins and solvent-detergent yields a final concentration of 0.3% TNBP (tri-n-butyl phosphate) and 1.0% Triton-X-100 and is incubated for about four hours at about 30°C.

Claim 34 (Previously presented) The method of claim 1, wherein the step of passing the concentrated purified extract solution containing immune globulins through a column containing C-18 resin for removal of remaining residue further comprises adjusting the pH of the concentrated purified extract solution containing immune globulins to about 4.6 to about 5.0 with a 4.0M sodium acetate buffer.

Claim 35 (Original) The method of claim 1, wherein the column containing the C-18 resin is equilibrated with a 20mM sodium acetate buffer at a pH of about 4.6 to about 5.0.

Claim 36 (Currently amended) The method of claim 1, wherein the ~~collected~~ concentrated purified extract solution containing immune globulins is formulated for final use in one of a liquid and a freeze-dried preparation.

Claim 37 (Currently amended) The method of claim 36, wherein the ~~collected~~ concentrated purified extract solution containing immune globulins is a liquid preparation with a formulation wherein the concentration of the immune globulins is adjusted to a range of about 5.0 to about 10.0 grams/deciliter protein, 0.1% polysorbate-80 (Tween-80), 0.2M glycine, and pH in a range of about 8.2 to about 8.6.

Claim 38 (Currently amended) A method for producing a high yield of purified immune globulins from blood plasma, comprising:

providing a plasma source containing immune globulins;

suspending the immune globulins in an ethanol solution at a volume equivalent to two times that of the plasma source at a temperature in a range of about -4°C to -6°C;

adjusting the pH of the suspension to about 5.7 to 5.8;

adding a volume of a solution of about 2.4M glycine in about 7% ethanol and purified water (volume/volume) equivalent to the volume of the plasma source to the suspension;

extracting the immune globulins from the suspension in a liquid phase to provide an extract containing immune globulins;

concentrating the extract containing immune globulins to provide an immune globulin solution of approximately 1.0 gram/deciliter immune globulins content;



performing solvent-exchange on the immune ~~globulins~~ globulin solution with a sodium phosphate solution to provide an extract solution containing immune globulins;

removing impurities from the extract solution containing immune globulins using an anion exchange chromatography column to provide a purified extract solution containing immune globulins;

concentrating the purified extract solution containing immune globulins deriving from an effluent of the anion exchange chromatography column to provide a concentrated purified extract solution containing immune globulins to provide a concentrated purified extract solution containing immune globulins;

inactivating viruses present in the concentrated purified extract solution containing immune globulins;

passing the concentrated purified extract solution containing immune globulins through a column containing C-18 resin for removal by adsorption of any residue in the concentrated purified extract solution containing immune globulins from the step of inactivating viruses, wherein the ratio of immune globulins load volume to resin volume is approximately eight parts load volume to one part C-18 resin; and

formulating the ~~collected~~ concentrated purified extract solution containing immune globulins for final use.